

5 **TISSUE ENGINEERED BIOMIMETIC HAIR FOLLICLE GRAFT**

CROSS-REFERENCE TO RELATED APPLICATIONS

Not Applicable

STATEMENT REGARDING FEDERALLY SPONSORED

10 **RESEARCH OR DEVELOPMENT**

Not Applicable

INTRODUCTION

Male pattern baldness is a common condition that is often treated by hair transplant surgery. In this procedure hair follicles from areas of the scalp that are not within the baldness pattern are excised and re-implanted within the baldness pattern to create the illusion of a fuller head of hair. No new hair is created by this procedure. Its success is limited by the number of follicles that can be harvested and re-implanted into the baldness pattern. Moreover, since not all explanted follicles are successfully transplanted, this technique, while the best available, has significant shortcomings.

20 It is well known that specific types of cells found in sub-structures within the hair follicle have the capacity to induce the formation of complete, normally functioning hair follicles. Such cells are known as follicular stem cells or follicle progenitor cells. In view of the large market for an effective hair restoration treatment, many attempts have been made to exploit the follicle-inducing capacity of these cells for the purpose of hair multiplication. Although apparently scientifically feasible, all schemes disclosed in the prior art have proven to fall short of the goal of providing a clinically and cosmetically acceptable new treatment for baldness. For example, cultured dermal papilla cells from a rat whisker were implanted just under the skin on a rat's ear, resulting in the growth of whiskers from the site of implantation. *Cf.*, R.F. Oliver and C.A.B. Jahoda in U.S. Patent 30 4,919,664, "Stimulation of hair growth", April 24, 1990, the teachings of which are incorporated by reference herein. In an unpublished clinical study of 5 human volunteers by Dr. Andrew Messenger, hair follicle dermal papilla cells dissected from scalp biopsies of the volunteers were cultured. The cells were multiplied and covered the surface of the culture flask, were removed by scraping them off the surface of the flask, and implanted

5 into shallow incisions in the skin on the underside of the forearm in each subject from whom the cells were obtained. No new hair growth was observed at the sites of cell implantation. All sites were biopsied after 6 months and the histological findings were normal with no evidence of scalp hair follicle formation.

10 More recently, T.H. Barrows described in International Publication WO 02/15952, "Scaffolds for Tissue Engineered Hair", February 28, 2002, the teachings of which are incorporated herein, a method of implanting the same type of cultured cells on a porous "scaffold" made from a bioabsorbable material. A subsequent clinical study in this case produced the first documented example of the induction of new hair follicles and hair growth in the skin of a human subject from the implantation of cultured cells
15 (see T.H. Barrows, S.A. Cochran, E.I. Griffin, and A.R. Solomon, "Tissue Engineered Human Hair: Preliminary Clinical Results", *TE2002: International Workshop on Tissue Engineering*, St. Gallen, Switzerland, 24-27 February, 2002, the teachings of which are incorporated by reference herein). Cells injected without the presence of a solid scaffold structure failed to show any evidence of follicle neogenesis. However, only one out of
20 16 implantations of cells in combination with scaffolds actually resulted in the growth of cosmetically useful hair shafts. Although equivocal evidence of follicle neogenesis in a number of the cell-plus-scaffold implant sites was noted histologically, there was also a persistent inflammatory response to residues of partially degraded scaffold debris. This type of foreign body response has been postulated to create a detrimental environment
25 for hair follicle formation (see K.S. Stenn, "Compositions and methods for inducing new hair follicle formation and hair growth in a desired orientation", U.S. Patent Application 10/123,984, April 17, 2002, the teachings of which are incorporated herein).

Thus there remains an unmet need for a reliable and reproducible method for culturing hair follicle progenitor cells and implanting the cultured cells into skin such
30 that new, cosmetically viable hair follicles are created.

SUMMARY OF THE INVENTION

Briefly, in one aspect, the present invention is an improved scaffold which is constructed to mimic the architecture of the native hair follicle and which is designed for
35 percutaneous implantation. Portions of the implant serve as supporting structures for the seeding of specific types of cells and other portions in contact with or protruding through

5 the epidermis serve as a site for epidermal down-growth. This down-growth is beneficial in creating an infundibulum in communication with the implanted cells, which in turn is helpful in controlling the angle of egress of the newly forming hair shaft.

In another aspect, the present invention is the use of specific compositions and methods of manufacture to produce scaffolds that combine biocompatibility with the
10 desired rates of bioabsorption. The performance characteristics of these types of scaffolds are critically important in the construction of a successful follicle-inducing implant, which facilitates or enhances the follicle neogenesis process.

In yet another aspect, the present invention is a specific combination of cultured cells that provide reliable and reproducible initiation of follicle neogenesis. The
15 implantation of cultured dermal papilla (also known as follicular papilla) cells alone gives unpredictable, non-reproducible results. It has been found that keratinocytes, suitably keratinocytes obtained from neonatal skin (*e.g.* infant foreskin tissue), also must be implanted in combination with the dermal papilla cells. Other types of cells optionally also can be combined with the dermal papilla/keratinocyte combination to
20 improve the success rate of follicle neogenesis. Optional additional cells may be selected from stem cell populations that are known to exist in human embryo, fetal or infant scalp skin, infant foreskin, umbilical cord blood, adult bone marrow, muscle, adipose tissue, and skin.

A further aspect of the present invention is the surprising discovery of
25 chondroitin-6-sulfate as a substance that provides a beneficial effect upon, or enhancement of, the follicle neogenesis process.

In another aspect, the present invention is a process for manufacturing a biomimetic hair follicle graft and a method for seeding it with cells and implanting it into skin where the growth of a new hair shaft is desired.

30 A further aspect of the present invention is a method for hair multiplication in which cells from harvested follicles, are multiplied in culture, and aliquoted into a multitude of bioabsorbable scaffolds in combination with cultured keratinocytes or other allogeneic cells. Implantation of the resultant cell-seeded biomimetic grafts provides a higher degree of hair restoration than possible by methods of the prior art. Thus the
35 method of the present invention reduces, inhibits or cures loss of hair specifically including, but not limited to, male or female pattern baldness and other hair loss conditions.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a cross-sectional schematic representation of a hollow filament of the present invention showing a solid outer filament (1) of bioabsorbable polymer, a porous inner filament (2) of the same or different bioabsorbable polymer, and a central lumen (3).

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Figure 2 is a cross-sectional schematic representation of the hollow filament of Figure 1 showing cells (4) (*e.g.* keratinocytes) that have been seeded into the porous inner filament by wicking a suspension of cells into the lumen of the filament and a clump of cells (5) (*e.g.* cultured dermal papilla cells or a hair follicle fragment) that has been seeded into the lumen of the filament by mechanically forcing it into an open end of the filament.

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Figure 3 is a cross-sectional schematic representation of the filament of Figure 2 shortly after implantation in the skin such that the proximal end (6) is in the dermis (7) and the distal end (8) is surrounded by down-grown epidermis (9).

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Figure 4 is a scanning electron micrograph (SEM) of a hollow filament of the present invention made of a porous copolymer of *d,l*-lactide and glycolide (PLGA).

Figure 5 is an SEM of the porous inner surface of the hollow filament of Figure 4, which was exposed by cutting open the tube.

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Figure 6 is a light photomicrograph showing a filament (10) with the construction shown in Figure 1 wherein the outer filament (1) is made of solid PLGA and the porous inner filament (2) is made of crosslinked hyaluronic acid (HAX). Figure 6 also shows a filament (11) made of PLGA containing no HAX. Both filaments were placed on a drop of water (12) colored red with food color dye. The PLGA only fiber (11) floated on the top of the water and did not wick any water into the lumen of the fiber, whereas the HAX-containing PLGA fiber (10) rapidly wicked the water into the lumen, giving the fiber a red color.

Figure 7(A) is a photograph of a mouse vibrissa (whisker) follicle (13) and a PLGA hollow filament (14) with an inside diameter of sufficient size to accommodate the excised follicle.

35 Figure 7(B) is a photograph of the PLGA hollow filament of Figure 7(A) with the follicle (13) inserted into the lumen.

5 Figure 8 is a photograph of a mouse whisker (15) that was observed growing on the back of a mouse against the background of regrown shaved pelage hair (16) 30 days post-implantation of a vibrissa follicle contained in a PLGA hollow filament.

 Figure 9 is a photomicrograph of a hair follicle bulb (17) and hair shaft (18) growing under the skin of a mouse 30 days post-implantation of a mixture of cells
10 obtained from neonatal mouse skin contained in a PLGA hollow filament (19).

 Figure 10 is a side-by-side comparison of two photomicrographs taken at the same magnification of the underside of skin excised from a nude mouse that had been injected 13 days previously with cells obtained from newborn black mouse epidermis and dermis. Panel A shows the control injection site and panel B shows the injection site
15 containing exactly the same number of cells except that the injection fluid also contained 5% (w/v) of chondroitin-6-sulfate, which resulted in the neogenesis of hair follicles that are larger and more numerous than the control.

 Figure 11 also is a side-by-side comparison of two photomicrographs taken at the same magnification of the underside of skin excised from a nude mouse that had been
20 injected 13 days previously with cells obtained from newborn black mouse epidermis and dermis. Panel A shows the same control injection site of Figure 10 and panel C shows the injection site in the same mouse containing exactly the same number of cells except that the injection fluid also contained 20% (w/v) of Pluronic™ F-127 surfactant (a copolymer of ethylene and propylene oxides), which resulted in the neogenesis of hair
25 follicles that are larger than those in the control.

 Figure 12 is a cross-sectional schematic representation of a hollow filament of the present invention in which the lumen (20) is slightly tapered to a closed end (21) attached to a porous plug (22).

 Figure 13 is a cross-sectional schematic representation of a hollow filament of the
30 present invention showing a fine pipette tip (23) containing cells (24) and fluid (25) that has been inserted into the tapered lumen (20).

 Figure 14 cross-sectional schematic representation of a hollow filament of the present invention showing a suspension of fluid (25) and cells (24) being expelled from a pipette tip (23) whereby the cells collect in the closed end of the lumen (21) and the fluid
35 (25) is fully absorbed in the porous plug (22).

 Figure 15 is a photograph of the hollow filament scaffold of Example 10 and a 10-microliter pipette tip (26) identical to the one that was used as a mandrel in the

5 process for making this embodiment of the present invention. The sheath (27) is made of PLGA and the fibrous mass (28) contained in the bulbous tip (29) is made of crosslinked gelatin/chondroitin-6-sulfate filaments. Charcoal particles (30) collected in the proximal portion of the fibrous scaffold upon injection of a slurry of charcoal particles and water into the scaffold through a pipette tip inserted into the scaffold sheath.

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DEFINITIONS

As used herein, the terms listed below shall have the following meanings:

“Tissue engineering” is defined as the art of creating combinations of cells, biocompatible scaffolds, usually bioabsorbable scaffolds, that have utility in replacing,
15 repairing, or augmenting tissues and organs of the human body.

“Follicle neogenesis” is defined as the phenomenon of new hair follicle formation in a region of the skin where none previously existed or in addition to and among pre-existing follicles.

“Bioabsorbable” is defined as the property of a material that allows it to be
20 broken down in the body into non-toxic by-products that are excreted from the body or metabolized therein.

“Scaffold” is defined as a non-cytotoxic structure that is capable of containing living cells and holding them in a specified configuration.

“Filament” is defined as a cylindrical structure that has a length that is greater
25 than its diameter.

“Fiber” is defined as a filament that possesses physical integrity.

“PLGA” is defined as a copolymer of lactide and glycolide.

“VEGF” is defined as vascular endothelial growth factor.

“EDC” is defined as N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide
30 hydrochloride.

“TFE” is defined as 2,2,2-trifluoroethanol.

“HAX” is defined as crosslinked hyaluronic acid.

“Mandrel” is defined as a cylindrical, tapered, or conical object used to hold the shape of materials applied to its outer surfaces and is removed in a process that yields a
35 hollow filament from said applied materials.

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DETAILED DESCRIPTION OF THE INVENTION

In an embodiment of the present invention, the scaffold component of the biomimetic hair follicle graft is a hollow filament comprised of one or more bioabsorbable polymers which define an interior lumen generally extending from one end of the scaffold to the other. The interior walls of the hollow filament or the exterior walls can be either smooth or porous depending upon the desired use. For example, a hollow filament with a relatively smooth surface can be used to retain one type of cell (e.g. human foreskin keratinocytes) on the luminal surface and another type of cell (e.g. cultured adult human hair follicle papilla cells, suitably in the form of an aggregated clump of cells), positioned within the lumen and in contact with the surrounding surface-attached cells. A hollow filament with a highly porous, hydrophilic interior can be used to wick a suspension of cells of one or more type into the filament, which by itself may be relatively hydrophobic and therefore not capable of wicking aqueous fluid.

The porous, hydrophilic interior may have a faster rate of bioabsorption or liquefaction than the exterior of the hollow filament to facilitate reorganization of the seeded cells within the lumen of the filament while continuing to be contained by the more slowly, bioabsorbing filament walls. Put otherwise, a scaffold polymer may have a variable or gradient of bioabsorptive rate from luminal interior to the scaffold exterior. Thus, interior *i.e.*, luminal, polymer bioabsorptive or bioabsorption rates are higher than exterior or externally-disposed polymer bioabsorptive rates.

Bioabsorbable, biocompatible materials suitable for both manufacture of hollow filaments and for use as a material for filling the interior of the hollow filaments can be selected from any of a wide variety of biocompatible, synthetic, natural, and semi-synthetic materials commonly used in clinical practice and in biomedical research. The hollow filaments may be comprised of polymer(s) including poly(lactic acid), poly(glycolic acid), poly(trimethylene carbonate), poly(dimethyltrimethylene carbonate), poly(amino acids)s, tyrosine-derived poly(carbonates)s, poly(carbonates)s, poly(caprolactone), poly(para-dioxanone), poly(esters)s, poly(ester-amides)s, poly(anhydrides)s, poly(ortho esters)s, collagen, gelatin, serum albumin, proteins, polysaccharides, mucopolysaccharides, carbohydrates, glycosaminoglycans, poly(ethylene glycols)s, poly(propylene glycols)s, poly(acrylate esters)s, poly(methacrylate esters)s, poly(vinyl alcohol), and copolymers, blends, and mixtures of

5 said polymers as well as oligomers containing bioabsorbable linkages that are block-copolymerized with otherwise non-degradable polymers that are metabolizable or excretable upon release by hydrolysis or degradation of said bioabsorbable linkages. Surface modification, graft polymerization, copolymerization, or blending of the bioabsorbable materials of this invention with growth factors, cell attachment binding
10 site moieties, and cell signaling molecules may be advantageous for improved cell attachment and/or improved cell function, aggregation, or initiation of the follicle neogenesis process.

Naturally occurring polymers (or biopolymers, or biomaterials) suitable for use as hollow filaments or as filling materials for hollow filaments include collagen, gelatin,
15 cellulose derivatives, starch, dextrin, chitosan, lipoproteins, recombinant human forms of collagen and gelatin, fibrinogen, fibrin, fibronectin, laminin, albumin, other serum proteins, polysaccharides, mucopolysaccharides, and other biopolymers that naturally occur in the human body. Suitable biopolymers can be used either in native form or in modified form such as by crosslinking with toxicologically acceptable crosslinking
20 agents *e.g.*, to reduce solubility. The filling material can be utilized in a variety of physical forms including fibers, gels, and porous structures. For example, cells can be combined with a solution of fibrinogen, which can then be converted into a bioabsorbable gel upon exposure to thrombin.

Another approach involves the use of a copolymer of ethylene oxide and
25 propylene oxide known as Pluronic™ F-127, which is commercially available from BASF Corp., Mount Olive, NJ. This surfactant is compatible with living cells and above a critical concentration forms a gel when warmed to body temperature from cooler temperatures. Thus the hollow filament can, for example, first be treated with a solution of Pluronic™ F-127 in alcohol followed by evaporation of the alcohol to impart a
30 hydrophilic coating on its luminal surfaces. A cold solution of Pluronic™ F-127 containing a suspension of cells can then be wicked or injected into the lumen of the filament and placed in a warm environment to gel the Pluronic™ F-127 and prevent the cells from being dislodged from the filament. Other biocompatible gel-forming materials include collagen, gelatin, serum albumin, Matrigel™ basement membrane matrix (BD
35 Biosciences, San Jose, CA) and various polyethylene glycol molecules with end groups that covalently react to form gel networks.

5 Other uses for the structures of the present invention are envisioned. For
example, by simply bundling together a large number of hollow filaments, with or
without the addition of other ingredients, it is possible to obtain a three dimensional
object as the finished product with continuous pores running through it. Such structures
are useful in the field of tissue-engineered cartilage. Because cartilage is essentially
10 avascular, scaffolds used for tissue-engineered cartilage suitably have an interior that is
readily accessible to nutrients. Moreover, the seeding of such scaffolds is facilitated by
pores coursing directly through the device. In addition, by orienting the pores along the
axis of the biomechanical load, seeded chondrocytes will be stimulated to respond
appropriately and organize into the columnar architecture of native articular cartilage.

15 Hyaluronic acid is known to be a useful biomaterial for tissue engineering
applications and is suitable for the above-mentioned scaffolds for tissue engineered
cartilage. A suitable material is obtained by self crosslinking hyaluronic acid with a
condensing agent, suitably EDC as described by K. Tomihata and Y. Ikada,
"Crosslinking of hyaluronic acid with water-soluble carbodiimide", *J. Biomed. Mater.*
20 *Res.*, 37, 243-251 (1997), the teachings of which are incorporated by reference herein.
Alternatively, hyaluronic acid can be crosslinked by a variety of other approaches
including methods described in "Modification of Natural Polymers: Hyaluronic Acid" by
Y. Luo, K. R. Kirker, and G. D. Prestwich, Chapter 45 in Methods of Tissue
Engineering, A. Atala and R.P. Lanza, eds., Academic Press, 2002, pp. 539-553, the
25 teachings of which are incorporated herein.

 Alternatively, hyaluronic acid can be converted into an insoluble material for use
in the present invention by esterification, as described in U.S. Patent 4,851,521, "Esters
of Hyaluronic Acid", by Francesco della Valle and Aurelio Romeo (July 25, 1989), the
teachings of which are incorporated herein. A suitable material of this type is the benzyl
30 ester of hyaluronic acid. While trans-esterification is a suitable method of crosslinking
because the resultant product is converted back into soluble hyaluronic acid upon
hydrolysis of the ester linkages within a few days *in vivo*, other crosslinking agents and
added crosslink-forming molecules also can be employed. Amine terminated
crosslinking molecules are also suitable, including, but not limited to, aliphatic diamines,
35 diaminoacid esters such as alkyl esters of lysine, and amine-terminated poly(ethylene
glycol).

5 Many of the chemical methods of crosslinking hyaluronic acid also can be used to facilitate covalent attachment of bioactive molecules to the hyaluronic acid structure to enhance the performance of the resultant scaffold. For example, peptides containing the cell attachment domain sequence of amino acids Arg-Gly-Asp (RGD) can be used to enhance cell attachment to the crosslinked hyaluronic acid scaffold.

10 In the use of hyaluronic acid to manufacture scaffolds for tissue engineered hair follicles it may be desirable to attach growth factors and angiogenesis factors to the scaffold in such a way as to be released during degradation of the scaffold for encouraging blood vessels to grow into the newly forming follicle or for other beneficial purposes. In addition to covalent attachment of small molecules to the scaffolds, higher
15 molecular weight molecules such as proteins, glycoproteins, and other biopolymers, such as collagen, laminin, fibronectin, and the like, can be physically or electro-statically bound into the structure to provide greater physical integrity, cell attachment capacity, or bioactivity. Other glycosaminoglycans including chondroitin sulfate, heparin, dermatan sulfate, versican, and the like also can be used advantageously in the present invention in
20 place of hyaluronic acid.

 A suitable material for manufacture of scaffolds for tissue engineered hair follicles is chondroitin-6-sulfate. The surprisingly beneficial effect of chondroitin-6-sulfate on the process of follicle neogenesis is illustrated in Example 6. A suitable composition is a mixture of chondroitin-6-sulfate and gelatin, crosslinked with EDC, and
25 rendered microporous with the use of sebacic acid particles as a porogen, as illustrated in Example 9, or by conversion into a mass of fibers as illustrated in Example 10.

 Hair follicle formation requires an interaction between cells of the epidermis (*i.e.* keratinocytes) with cells of the dermis (*i.e.* dermal fibroblasts or dermal/follicular papilla cells). Dermal and epidermal cells for use in the manufacture of tissue engineered living
30 skin equivalents for use in the treatment of burn patients are often currently obtained from human infant foreskin tissue because it is readily available. These skin equivalents, although of great clinical value, are devoid of hair follicles. Thus keratinocytes generally or keratinocytes from that source have generally not been thought to have any particular value in the construction of tissue-engineered implants that are required to induce the
35 formation of new hair follicles. It has been stated that, “[p]referably the epidermal cells are from the same patient being treated...” in the disclosure by Cooley and Vogel (WO 99/01034), the teachings of which are incorporated herein.

5 Surprisingly, it has been discovered here that keratinocytes from the adult scalp
are not particularly reliable and that keratinocytes from the infant foreskin are, in fact,
more effective in their ability to induce follicle neogenesis in combination with dermal
papilla cells. While the reason for this finding is unknown (and not wishing to be bound
by any particular theory), we believe that epidermal stem cells, a very small
10 subpopulation of epidermal keratinocytes, are required to initiate follicle neogenesis and
that infant epidermis is a richer source of these cells than adult epidermis. Other sources
of stem cells from the infant, therefore, also could be useful. For example, blood
obtained from the umbilical cord could be a source of useful stem cells, as could cells
obtained from embryos or established embryonic stem cell lines, or cells obtained from
15 infant scalp skin under established organ donor procedures.

The dermal component of the epidermal/dermal cell construct of the present
invention can be obtained from dissected follicles obtained from the subject who is to
receive the hair restoration treatment. Another possibility, however, is to obtain cells
from the scalp of an organ donor, suitably an infant. Cells that are to be seeded into a
20 scaffold can be obtained by culture of a follicle fragment selected from fragments
comprising the dermal papilla, dermal sheath, matrix, and inner and outer root sheaths.
Alternatively, cells can be simply cultured from a mixture of cells prepared from the
intact scalp dermis or from scraps of dermal and follicular tissue remaining after
follicular unit dissection of donor site tissue from a traditional hair restoration surgical
25 procedure in which the donor is also the recipient of said cells.

Epidermal cells, regardless of their origin, are suitably contained within the
lumen of the hollow filament, adherent to or adjacent to the lumen interior wall or
otherwise contained in a porous structure therein. Hair follicle progenitor cells obtained
from a biopsy of hair bearing scalp, and multiplied in culture, from the person who is to
30 receive the biomimetic grafts are suitably contained primarily in the proximal
compartment of the filament lumen, which corresponds to the bulb of a hair follicle.
Thus the graft is self-contained in that both dermal and epidermal components are
present in the correct relationship to each other, obviating the need for implanted cells to
interact with the intact epidermis or epidermal cells that grow down the implanted graft.
35 This, in turn, provides reliability and reproducibility of new hair follicle formation.

Alternatively, dermal and epidermal cells can be combined as a mixture, with the
optional addition of stem cells, and seeded into the scaffold just prior to implantation. In

5 this case the cells would associate and reorganize *in situ*. A suitable scaffold
configuration for this method of cell delivery is shown in Figures 12 through 14. In this
embodiment, the scaffold is manufactured as a hollow filament with one end closed to
form a porous, sponge-like structure that serves as a reservoir to absorb fluid. Thus upon
injection of a suspension of cells and fluid into the lumen of the hollow filament, the
10 fluid is wicked into the reservoir end causing collection of the cells into a concentrated
area at the closed end of the filament. The scaffold containing cells is then immediately
implanted into a stab wound in the skin in the same manner that follicular grafts are
implanted in a traditional hair restoration surgery procedure. A process for
manufacturing the scaffold of this embodiment is illustrated in Example 9 and comprises
15 the following steps:

1. Provide a tapered mandrel with the same dimensions as the distal segment of a
pipette tip or other suitable fluid delivery means.
2. Provide a mold cavity, open at both ends, that accommodates the mandrel of step
1 as an insert for the cavity, which is longer than the mandrel, the extra length
20 creating a void volume approximately equal to the volume of the mandrel itself.
3. Provide a mixture of bioabsorbable materials A dissolved in solvent B and
porogen particles C that are soluble in solvent D, but not in solvent B.
4. Fill the cavity of step 2 with the mixture of step 3 and insert the mandrel of step
1.
- 25 5. Remove solvent B and extract the molded part from the mold cavity.
6. Place the molded part of step 5 into a solvent D to remove the porogen particle.
7. Place the molded part of step 6 into a solution of crosslinker E dissolved in
solvent F.
8. Rinse with fresh solvent F or other suitable solvent and allow the resultant
30 scaffold to dry.
9. Optionally re-hydrate the scaffold of step 8 with an aqueous solution of a water-
soluble substance G and allow the water to evaporate to create a protective
coating.
10. Prepare a solution of bioabsorbable material H in solvent I.
- 35 11. Coat the scaffold of step 9 with the solution of step 10 and allow solvent I to
evaporate.

- 5 12. Remove the protective coating of material G by soaking the scaffold in water, rinsing with fresh water, and allowing the water to evaporate.

In a suitable process involving the above steps, A is a mixture of chondroitin-6-sulfate and gelatin, B is water, C is sebacic acid particles less than 63 microns, D is acetone, E is EDC, and F is a 9:1 mixture by volume of acetone:water.

- 10 A modification of the above process to create the embodiment illustrated in Example 10 comprises the following steps:

1. Provide a tapered mandrel with the same dimensions as the distal segment of a pipette tip or other suitable fluid delivery means except that the tip of said mandrel is extended slightly to provide a sharp point.
- 15 2. Coat the mandrel of step 1 with a water-soluble polymer A.
3. Attach to the tip of the mandrel of step 1 a permeable, bioabsorbable filtration substance B that is suitable for separation of cells from a suspension of cells in fluid, said filtration substance being substantially encased in a removable protective coating C soluble in solvent D.
- 20 4. Coat the entire mandrel and said filtration substance B with a film-forming bioabsorbable polymer E dissolved in solvent F.
5. Remove solvent F of step 4 to create a continuous layer of bioabsorbable polymer film covering the mandrel and attached tip of step 3.
6. Create an opening in the film of step 5 by removing a small piece of said film covering the filtration material distal to its point of attachment to the mandrel.
- 25 7. Substantially remove polymer A by soaking the product of step 6 in water.
8. Substantially remove the protective coating material C by soaking in solvent D.
9. Remove the mandrel from the product and continue steps 7 and 8 as necessary to fully remove materials A and C.

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In a suitable embodiment of the above process, A is a surfactant, for example Pluronic™ F-127, B is a mass of fibers comprised of chondroitin-6-sulfate and cross-linked gelatin, C is caramelized cane sugar, D is water, E is PLGA, and F is dichloromethane.

- 35 A suitable method for manufacturing long hollow filament scaffolds of the present invention that are open at both ends involves the use of nylon monofilament as a mandrel, subsequently removed by dissolution, upon which the various ingredients are

5 deposited. Fibers made from other materials that can be removed without damaging the biomaterial investments are also envisioned as being useful in this process. Alternatively, hollow filament melt extrusion is a proven technology that also can be used to create the hollow filaments of the present invention.

10 In a process of the present invention, a fine nylon fiber is first coated with particles to ensure that application of a bioabsorbable material in the form of a viscous solution will adhere to the fiber. These particles are applied to, adhered to, or associated with, the nylon fiber using a solution of nylon or other polymers such as polystyrene. The nylon (or other polymer) particles firmly adhere to the fine nylon fiber and assist the subsequent coating of the fiber with a viscous, aqueous solution, for example a solution
15 of hyaluronic acid, which otherwise would not cling to the nylon surface. A suitable substance for use as particles is sebacic acid, which is substantially insoluble in water (a solvent for hyaluronic acid) and TFE (a solvent for nylon), but is freely soluble in acetone (a non-solvent for hyaluronic acid and nylon). The particles also serve to impart a porous lumenal surface on the resultant hollow filament upon removal of said particles.
20 After curing, crosslinking, or otherwise rendering the coated bioabsorbable material insoluble in water, the nylon and the particles attached to the nylon are removed with the appropriate solvents. If desired, an additional coating of a different bioabsorbable material may then be applied. At this stage of the process, with the nylon and nylon-bound particles removed, a wider choice of solvents can be used to apply this second
25 material. A suitable second material is a copolymer of lactide and glycolide (hereinafter referred to as PLGA), thereby providing in this process a hollow filament with a HAX interior and a PLGA exterior.

From the above, the detailed steps of this process are:

1. Provide a water-soluble material W.
- 30 2. Provide a fiber of material X that is soluble in solvent A.
3. Provide bioabsorbable material Y that is soluble in solvent B.
4. Provide particles P that are soluble in solvent C.
5. Provide bioabsorbable material Z that is soluble in solvent D.
6. Provide a polymer M that is soluble in solvent N.
- 35 7. Prepare a solution of polymer M in solvent N.
8. Coat the fiber of step 2 with the solution of step 7.

- 5 9. Coat the particle P of step 4 onto the coated fiber of step 8 and allow solvent N to evaporate, thereby bonding said particles onto said fiber.
10. Prepare a solution of material Y in solvent B.
11. Coat the solution of step 10 onto the particle encrusted fiber of step 9 and allow solvent B to evaporate.
- 10 12. If material Y is water soluble, render material Y water insoluble by crosslinking.
13. Remove polymer M from the coated fiber of step 12 by dissolution and rinsing with solvent N.
14. Remove the particles P from the coated fiber of step 13 by dissolution and rinsing with solvent C.
- 15 15. Remove the fiber X from the coated fiber of step 14 by dissolution and rinsing with solvent A.
16. Prepare a solution of material W in water.
17. If required, apply a protective coating of the solution of step 16 to the filament of step 15 and allow the water to evaporate.
- 20 18. Prepare a solution of bioabsorbable material Z in solvent D.
19. Coat the fiber of step 17 with the solution of step 18 and allow solvent D to evaporate.
20. Remove the protective coating of material W by soaking the filaments in water, rinsing with fresh water, and allowing the filament to dry.
- 25 21. Cut the filaments into the appropriate lengths and sterilize.
22. Seed the filaments with the appropriate cells by allowing a suspension of the cells in the appropriate volume of the appropriate fluid to wick into the lumen of the filament.
23. Optionally load the filament with additional cellular constructs by inserting into one end of the filament.
- 30 24. Make an incision in the skin where the growth of a hair shaft is desired and implant the filament of step 22 or 23 into the incision with the end containing a cellular construct imbedded in the skin and the distal end extending percutaneously above the skin.
- 35 In a suitable process involving the above steps, W is caramelized cane sugar, X is nylon, A is TFE, Y is hyaluronic acid (sodium salt), B is water, P is sebacic acid (particle size 10 to 500 microns, preferable 50 to 200 microns), Z is PLGA, D and N are

5 dichloromethane, M is polystyrene, and C is acetone. If desired to impart porosity to the PLGA, D can be specified as a solution of glycerol and TFE. Upon evaporation of the TFE, the PLGA and glycerol phase separate into a bicontinuous emulsion. Removal of the glycerol by dissolution in water imparts a microporous structure to the residual PLGA coating.

10 In order to manufacture a scaffold for use in cartilage tissue engineering applications, a large number of the fibers of step 11 above, with or without the addition of other ingredients, can be bundled together using additional coating solution of step 10 as an adhesive. Prior to dissolving the mandrel fibers the bundle can be cut into discs from which the desired products can be constructed after dissolving the fibers and
15 completing the processing steps.

EXAMPLES

Example 1. PLGA does not impair transplanted follicle survival and hair growth.

A copolymer (PLGA) of *d,l*-lactide and glycolide (52:48) was obtained from CCA Purac Biochem bv, Gorinchem, The Netherlands (Purasorb® PLGA, inherent
20 viscosity 1.06 dl/g in chloroform) and dissolved in dichloromethane (10% w/v). Cane sugar was melted and heated until caramelized and allowed to cool to the point that filaments of the desired size could be pulled from the melt. The filaments were cooled until solidified and then immediately placed on a surface covered with powdered sodium chloride to prevent them from becoming sticky. Hollow filaments were prepared by
25 coating the PLGA solution onto the filaments of powdered, salt-encrusted caramelized sugar. The dichloromethane was evaporated, and the sugar and salt were dissolved and removed by placing the coated filaments in water. Vibrissa (whisker) follicles were excised from euthanized C57Bl6 mice (Charles River) at Mercer University (Atlanta, GA) under an IACUC-approved. The excised whiskers were reimplanted into oblique
30 incisions on the shaved dorsal skin of syngeneic mice. This procedure was then repeated with the excised follicles first inserted and snugly fit into the lumen of the hollow PLGA filaments such that the follicles were completely surrounded by the polymer (Fig. 7). After 30 days the implanted mice were euthanized and the skin excised, stretched over rectangles of cardboard, fixed in formalin and processed by routine methods of paraffin
35 embedding, sectioning, and H&E staining for analysis by light microscopy.

After 30 days 7 out of 8 control follicle implants were found to be growing vibrissa hair shafts. 2 out of 3 PLGA/follicle implants also were found to be growing hair

5 shafts as well (Fig. 8). Histology did not reveal any abnormalities in the regenerated, transplanted follicles with or without ensheathment in PLGA.

Example 2. Preparation of microporous PLGA hollow filament.

A solution of glycerol (glycerine U.S.P.) and PLGA (Resomer™ RG504, Boehringer Ingelheim, Germany) was prepared in TFE (Aldrich Chemical Co., Milwaukee, WI) such that the ratio of glycerol to PLGA was 80:20 (w/w). This solution
10 was coated onto caramelized cane sugar filaments (prepared as described above in Example 1) in several layers with time allowed between coats for drying. Evaporation of the TFE, a solvent for both glycerol and PLGA, caused the concentration of the solutes to increase until glycerol no longer remained miscible. Phase separation of glycerol from
15 the PLGA/TFE resulted in the formation of a bicontinuous emulsion. The coated filaments were then placed in water, which caused the sugar and the glycerol, as well as any residual TFE, to dissolve rapidly and leach out of the now micro-porous PLGA. The resultant porous hollow filaments were rinsed with water and allowed to dry completely in a desiccator. The porous structure of the dry PLGA hollow filaments is shown in the
20 SEM photographs presented in Figures 4 and 5.

Example 3. Preparation of a hydrophilic PLGA hollow filament.

A solution of approximately 5% (w/v) Pluronic™ F-127 surfactant (Sigma Chemical Co., St. Louis, MO), which is a copolymer of ethylene oxide and propylene oxide, was prepared in anhydrous ethanol with gentle heating. Hollow filaments of
25 PLGA were prepared as described in Example 1. The clear, colorless solution of Pluronic™ F-127 was allowed to wick into one of the PLGA filaments and the ethanol allowed to evaporate completely. A drop of red food coloring dye was placed on a glass plate. A 15mm length of the Pluronic™ F-127-treated PLGA hollow filament and a 15mm length of the untreated control filament of the same diameter were contacted with
30 the dye by touching one end of each filament to the surface of the liquid. The Pluronic™ F-127-treated filament rapidly wicked the aqueous dye solution through its entire length whereas the untreated filament only slowly wicked the dye into 4mm of its length.

Example 4. Preparation of a PLGA coated HAX filament.

Sebacic acid (Aldrich Chemical Co., Milwaukee, WI) was ground by mortar and
35 pestle and sieved to obtain particles >63 and <212 microns in size. A one meter length of fine monofilament nylon fiber (0.003-inch diameter, Shakespeare Monofilament Division, Columbia, SC) was coated with a solution of nylon in TFE (10% w/v) and

5 immediately encrusted with the sebacic acid particles by drawing the fiber through a
pipette containing the solution and then through a pile of the sebacic acid particles placed
at the exit of the pipette tip. The fiber was suspended at one end and allowed to hang in a
vertical position. A solution of hyaluronic acid sodium salt was made by combining 180
mg of sodium hyaluronate powder (1.4 million molecular weight, Lifecore Biomedical,
10 Chaska, MN) with 10 ml of water and allowing this to hydrate and dissolve at room
temperature overnight. The solution was mixed well and then coated onto the fiber by
manually running a bead of the gelatinous liquid down the fiber. The thin coating of
hyaluronate solution was allowed to dry and then two additional coats were applied with
time allowed between coats for drying. The fiber was then cut into approximately 4-cm
15 lengths and placed in a vial containing 0.2% (w/v) of N,N'-
isopropylethylaminocarbodiimide (Sigma Chemical Co., St. Louis, MO) in acetone
containing 10% (v/v) water. After 3 hours at room temperature, the solution was
decanted and replaced with pure acetone. The acetone was decanted and residual acetone
removed by evaporation. The vial was then filled with TFE (Aldrich Chemical Co.,
20 Milwaukee, WI) and allowed to stand overnight at room temperature. The TFE was
decanted and replaced with fresh TFE and after several hours this was decanted and
replaced with acetone. The acetone was decanted and residual acetone removed by
evaporation to yield HAX filaments.

Cane sugar was heated over an open flame in a test tube with stirring until it
25 melted and caramelized. The dark brown melt was poured out and allowed to solidify.
A syrup was prepared by dissolving this in water (approximately 20% w/v). The dry
HAX filaments were placed in the syrup whereupon they rapidly absorbed the liquid and
became noticeable larger in diameter. The filaments were removed from the syrup and
allowed to dry partially. They were then coated with powdered sodium chloride (<63
30 microns) and placed in a desiccator to dry overnight. The stiff, brittle, amber colored
filaments were dipped into a 10% (w/v) solution of PLGA (Purasorb® PDLG, inherent
viscosity 1.06 dl/g in chloroform, CCA Purac Biochem bv, Gorinchem, The Netherlands)
in dichloromethane (Aldrich Chemical Co., Milwaukee, WI) and the solvent allowed to
evaporate. The coated filaments were then allowed to soak in water until the sugar and
35 salt dissolved, as evidenced by the loss of the amber color. The filaments were then
returned to the desiccator and allowed to dry completely. Control hollow filaments of
PLGA not containing HAX were prepared by the method of Example 1. The ability of

5 these filaments to wick aqueous fluid was tested by dipping one end into a drop of water containing red food coloring dye. The red colored water rapidly entered the lumen of the filament containing HAX and filled it completely, thereby imparting a red color to the filament. The filament not containing HAX, however, did not wick water and, being water repellant, floated on the surface of the water.

10 **Example 5.** Hair follicle neogenesis and hair shaft growth *in vivo* from mouse cells contained in a PLGA hollow filament scaffold.

Epidermal and dermal cells were isolated from newborn mouse pups as previously described (S.M Prouty, L. Lawrence, *et al.* (1996). "Fibroblast-dependent induction of a murine skin lesion with similarity to human common blue nevus." Am J Pathol 148(6): 1871-85), the teachings of which are incorporated herein. Two
15 deviations from the published procedure were the use of C57/Bl6 mice (Charles River Laboratories) and the use of dispase (Gibco), rather than trypsin, to facilitate separation of the epidermis from the dermis of the neonatal mouse skin. Once both populations of cells, dermal and epidermal, were separately isolated they were recombined so that the
20 ratio of dermal cells to epidermides, also known as epidermal buds, was 100:1. This cell combination was then spun down at 900 rpms for 5 minutes. The resultant pellet was resuspended in PBS to obtain a cell concentration of at least 10 million total cells per milliliter and promptly loaded into PLGA hollow filaments, prepared according to the method discussed in Example 1, by simply submerging the fiber in the suspension of
25 cells. These cell-seeded filaments were then implanted underneath the dorsal skin of a nude (Nu/Nu) mice by puncturing the skin with a 19-gauge hypodermic needle, withdrawing the needle until half of the open bevel was exposed, inserting the hollow filament implant into the opening, and then withdrawing the needle completely while pushing the filament under the skin. At three weeks post-implantation the mice were
30 necropsied and the skin excised. Follicle-like structures and hair shafts were observed associated with the PLGA scaffold material on the subcutaneous side of the skin at the site of filament implantation. As shown in Figure 9, a well-formed hair follicle bulb and long hair shaft can be seen in this photomicrograph. The scaffold material is difficult to visualize because it is colorless and partially degraded.

35 **Example 6.** Use of chondroitin-6-sulfate as additive in cell suspension.

The methods described in Example 5 were utilized to evaluate a number of soluble materials added to the suspension of cells prior to injection into the nude mouse.

5 Exactly the same number of each cell type was injected in both control and test material injection sites, which were located on the same mouse. As shown in Figure 10, the follicles formed in the subcutaneous space at 13 days post-injection of cells were clearly larger at exactly the same magnification in the presence of an initial 5% concentration of chondroitin-6-sulfate (Sigma Chemical Co., St. Louis, MO).

10 **Example 7.** Use of Pluronic™ F-127 as additive in cell suspension.

The experiment of Example 6 was repeated with the substitution of 20% Pluronic™ F-127 surfactant (Sigma Chemical Co., St. Louis, MO) for the chondroitin-6-sulfate. As shown in Figure 11, the follicles formed in the subcutaneous space at 13 days post-injection of cells were clearly larger at exactly the same magnification than the
15 follicles in the control injection site where no Pluronic™ F-127 surfactant was present in the injected fluid.

Example 8. Preparation of crosslinked gelatin hollow filaments.

Porcine skin gelatin (300 bloom, Sigma Chemical Co., St. Louis, MO) was dissolved in warm water to give a 5% (w/v) solution. This solution was applied to a fine
20 nylon filament encrusted with sebacic acid particles as described in Example 4. Several coats were applied with time allow between applications for the coatings to dry. The fiber was then cut into approximately 4-cm lengths and placed in a vial containing 0.2% (w/v) of EDC (Sigma Chemical Co., St. Louis, MO) in acetone containing 10% (v/v) water. After 3 hours at room temperature, the solution was decanted and replaced with
25 pure acetone. The acetone was decanted and residual acetone removed by evaporation. The vial was then filled with TFE (Aldrich Chemical Co., Milwaukee, WI) and allowed to stand overnight at room temperature. The TFE was decanted and replaced with fresh TFE and after several hours this was decanted and replaced with acetone. The acetone was decanted and residual acetone removed by evaporation to yield water-insoluble
30 gelatin hollow filaments.

Example 9. Preparation of crosslinked chondroitin-6-sulfate/gelatin scaffolds.

Sebacic acid (Sigma Chemical Co., St. Louis, MO) was ground with mortar and pestle into particles that passed through a 63-micron sieve. Disposable 10 microliter pipette tips (Eppendorf epTIPS™, Brinkman Instruments, Westbury, NY) were cut into
35 three equal pieces. The proximal piece was discarded and the middle piece was packed with the sebacic acid powder. Chondroitin-6-sulfate (80 mg) and porcine skin gelatin (80 mg) were dissolved in 2.0 ml of warm water. This solution was loaded into a syringe

5 fitted with a stainless steel tube that just fit into the larger opening of the sebacic acid filled pipette tip section. The solution was then injected into the packed sebacic acid such that air was expelled from the powder as the fluid moved through the packing. The distal piece cut from the pipette tip was then inserted into the filled piece. Excess paste that extruded out the open end was wiped off.

10 The assembled pieces were allowed to dry completely by storage in a desiccator overnight. They were then placed in a 0.2% (w/v) solution of EDC in 9:1 (v/v) acetone:water. After about one hour the two pieces of pipette tip were gently pulled apart and the molded product was ejected and returned to the EDC solution for an additional 3 hours, whereupon the crosslinked scaffold was soaked in pure acetone for
15 one hour, and then allowed to dry.

Example 10. Pipette tip sheath scaffold of PLGA and crosslinked gelatin/chondroitin-6-filaments.

Chondroitin-6-sulfate and porcine skin gelatin (100 mg each) were dissolved in 2.0 ml of warm de-ionized water and slowing injected through a 26-gauge needle into a
20 silicone rubber tube filled with acetone that was flowing from an elevated reservoir into a beaker. The resultant fine, white filaments were collected and placed in a 0.2% (w/v) solution of EDC in 9:1 (v/v) acetone:water for about 4 hours. The filaments were then rinsed with pure acetone and allowed to dry. A solution of 30% (w/v) caramelized cane sugar in water was added to the dry filaments and allowed to soak until the filaments
25 were completely saturated. A small tuft of the saturated fibrous mass was placed in a 1.0 mm inside diameter Teflon™ tube and allowed to dry completely in a desiccator. The cylinder of brown sugar encased filaments was ejected from the tube and mounted on the tip of a 30-gauge needle just protruding through the end of an Eppendorf pipette tip (10 microliter epTIPS™, Brinkman Instruments, Inc., USA). The pipette tip with attached
30 sugar encased filaments was dipped into a 30% (w/v) solution of Pluronic™ F-127 in dichloromethane, allowed to dry, and then dipped into a 15% (w/v) solution of PLGA in dichloromethane and allowed to dry, with the tip suspended point down. The distal end of the dried PLGA was cut off with scissors and the entire pipette tip was placed in water. After a few minutes the PLGA film was easily slipped off the pipette tip due to the
35 hydrated Pluronic™ F-127 surfactant coating. The resultant scaffold was allowed to soak further until all the brown color of the caramelized sugar disappeared.

5 To test the ability of the fibrous material contained in the PLGA sheath to function as filtration media, and therefore as a means of collecting and implanting hair follicle inductive cells, a slurry of charcoal particles (100-400 mesh, Norit CA1 Activated Charcoal, Sigma Chemical Co., St. Louis, MO) in water was drawn up into a 10-microliter pipette tip, the tip inserted into the PLGA scaffold sheath, and the slurry
10 ejected out of the pipette and out through the end of the scaffold. As shown in Figure 15, the charcoal particles were clearly visible in the proximal end of the fibrous material where they collected as the water passed through.

Example 11. Bundled hollow filament HAX scaffold for tissue engineered cartilage.

 Sodium hyaluronate (MW 1.4×10^6 Daltons, product no. 80081, LifeCore
15 Biomedical, Inc., Chaska, MN 55318) was dissolved in deionized water, placed in dialysis tubing and dialyzed against cation exchange cellulose (Dowex AG 50W-X4, purchased from BIO-RAD Laboratories, Inc., Hercules, CA) at 1 gram per 100 ml of deionized water and stirred by a magnetic bar at 4 °C for 2 days. The solution was removed from the dialysis tubing and freeze-dried to obtain a fluffy white solid, which
20 was re-dissolved in deionized water to obtain a viscous solution containing 3.4% (w/v) hyaluronic acid.

 Nylon monofilament thread (0.003 inches in diameter, SN-38 WonderThread™, Shakespeare Monofilament Division, Columbia, SC) was strung up in 3-meter lengths and coated with a slurry of sebacic acid powder (<63 microns) in a solution of
25 approximately 5% (w/v) polystyrene (broken disposable culture dish fragments) in dichloromethane by threading the fiber through a disposable transfer pipette (cat. no. 231, Samco Scientific Corp., San Fernando, CA) with a portion of the bulb cut off, injecting the slurry into the pipette through the bulb opening, and then running the pipette down the length of the fiber (bulb first) such that the pipette tip acted as an orifice for
30 uniform slurry deposition. Upon evaporation of the dichloromethane the coated fiber was flat white in color and had a noticeably rougher feel than the uncoated fiber. The above hyaluronic acid solution was then applied to the coated fiber in a similar manner. Five coats were applied with approximately one hour of drying time between the applications of each coat of hyaluronic acid. The fibers were then carefully coiled up and
35 placed in a covered dish containing a 0.2 % (w/v) solution of EDC in acetone containing 10% (v/v) deionized water and allowed to soak overnight while submersion in this liquid. They were then rinsed in pure acetone and strung up again, this time bundling

5 approximately 10 fibers together into one continuous tow. The tow was coated with
hyaluronic acid solution by hand, *i.e.* by holding a dab of the viscous solution between
the thumb and first two fingers of one hand with the tow positioned in the gap formed at
the juncture of the three digits and running the hand down the length of the tow. Several
coats were applied with time allowed for drying between each application. The coated
10 tow was then coiled and placed in a covered dish of EDC solution as above and allowed
to soak overnight. It was then rinsed with pure acetone and allowed to dry.

The tow was cut into 4 cm lengths and each piece was coated with a liberal
amount of hyaluronic acid solution. The coated pieces were bundled together and then
lashed with 0.009-inch diameter nylon monofilament (Shakespeare) by tying individual
15 loops around the bundle. As each loop was tightened and tied, excess hyaluronic acid
solution extruded out of the bundle, which was then hung up by one end to dry. Excess
solution dripped off the end and after one day the bundle was a hard, lightweight
composite. It was then cut into 3mm diameter discs with a razor blade and the discs were
placed in a fresh batch of the above-mentioned EDC solution overnight both to cross-link
20 the hyaluronic acid and to dissolve out the sebacic acid and in dichloromethane for
another overnight period to finish dissolving out the polystyrene. They were then placed
in TFE to dissolve out the nylon. The TFE was replaced after one hour with fresh TFE
and the discs were allowed to soak in this TFE overnight. They were then rinsed with
fresh TFE, then with acetone and allowed to dry. The dry discs were placed in a 50%
25 aqueous solution of glutaraldehyde (Acros no. 41096-5000, purchased from Fisher
Scientific Co., Fairlawn, NJ) and allowed to soak for 72 hours at room temperature as
recommended in "Polypeptide resurfacing method improves fibroblast's adhesion to
hyaluronan strands" by M. Hu, E.E. Sabelman, S. Lai, E.K. Timek, F. Zhang, V. R.
Hentz, and W. C. Lineaweaver, in *Journal of Biomedical Materials Research*, vol. 47
30 pages 79-84 (1999), the teachings of which are incorporated herein. This treatment
converted the EDC crosslinked HAX into a slower degrading material better suited for
the tissue engineered cartilage application. Upon removal of the discs from the
glutaraldehyde they were rinsed with several changes of deionized water and then
allowed to soak in water overnight. They were then rinsed again and placed in 70% (v/v)
35 isopropanol/water solution as a disinfectant prior to use for cell-seeding and bioreactor
studies.